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Effects of Drought Stress on Growth of Mulberry (*Morus alba* L.) Trees in the Hydro-Fluctuation Belt of the Three Gorges Reservoir Area*

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Abstract: To examine possible effects of soil water conditions in summer in the hydro-fluctuation belt of the Three Gorges Reservoir Area on mulberry, we grew potted mulberry in a greenhouse under three soil water regimes designed to simulate normal irrigation (CK), moderate drought stress (T1) and elevated drought stress (T2). The results showed the following. 1) With drought stress increasing, the heights, base diameters, and root surface areas of mulberry trees decreased significantly relative to the corresponding earlier period. The root/shoot ratio increased gradually in the earlier stage, and decreased gradually in the later stage. The specific root area of mulberry trees on the whole increased significantly relative to the corresponding earlier period. 2) With the elongation of treatment time, the growth of mulberry seedlings decreased gradually, and the heights, base diameters, root surface area and root/shoot ratio of the mulberry trees in the three treatment groups increased to different degrees. The specific root area of mulberry trees on the whole decreased gradually. We concluded that under the different degrees of drought stress the growth of mulberry trees would be inhibited, but the plants were also able to adapt to the stress, thereby maintaining physiological and metabolic viability. Biomass appeared to be re-allocated such that water absorption capacity was improved. Accordingly it can be concluded that the mulberry trees demonstrated potential for resisting and tolerating drought stress.

Key words: drought stress; hydro-fluctuation belt of Three Gorges Reservoir Area; mulberry trees; growth
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At present, many foreign and domestic restoration ecology specialists consider that plantations are among the most viable management approaches for the rehabilitation of injured ecosystems of hydro-fluctuation and riverside belts[1-6]. Selection of suitable plants is therefore a priority of revegetation science[7]. Faced with the counter-seasonal fluctuation of water levels and a significant drop of 30 m within a short period of time in the Three Gorges Reservoir, almost all the revegetation research in the region has focused on understanding the phenomenon of adaptation to waterlogging, and on the selection of plant species that tolerate waterlogging [8-10]. Nevertheless, there are also other problems to consider, notably that high temperatures and drought are frequent in the summer in the Three Gorges Reservoir Area[11]. Historically, especially in Chongqing, high temperature and summer drought events have occurred every 4 years out of 10, and the frequency is increasing. As a result many plants do not survive the summer drought period in the Three Gorges Reservoir Area. Thus, plants selected for revegetation of the hydro-fluctuation belt of the Three Gorges Reservoir should not only tolerate submergence but also be able resist summer drought.

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In recent years, the mulberry (*Morus alba* L.) has become a subject of considerable interest for its ecological functions, namely, adaptability to harsh natural environmental conditions, soil and water conservation, and improvement of air quality[12-15]. He et al[14] conducted investigations in the Three Gorges Reservoir Area and found that mulberry trees grew well during the severe drought in 2006, and on the whole did not die, showing strong drought resistance. However, little research has been conducted on the mechanism of drought and flooding tolerance of mulberry plants. Mulberry has mostly been planted for silkworm forage and its potential ecological roles have hardly been examined, nor has the species been used in ecological restoration work. Accordingly, here we describe experiments in which we simulate various soil water summer drought conditions that may occur in the Three Gorges Reservoir Area, and study the effects of drought stress on the growth of mulberry seedlings. We reveal the growth and adaptability of mulberry seedlings under drought stress conditions with a view to providing a theoretical basis for the selection of plants suitable for revegetation of the hydro-fluctuation belt of the Three Gorges Reservoir Area.

1 Materials and Methods

1.1 Experimental materials and methods

The experiment was begun with the germination of Guisangyou 12 mulberry seeds in a greenhouse at Southwest University on July 1, 2011. The mulberry seeds were provided by the silkworm station in Nantuo town in Fuling in the Three Gorges Reservoir Area. On August 1, 2011, mulberry seedlings of similar size were transplanted to pots (25 cm high and 20 cm aperture), each with 5 kg purple soil derived from the Three Gorges Reservoir Area, one plant per pot, and 120 pots in total. The seedlings were given 10 days to recover from transplanting, then they were divided into 3 groups randomly (each group containing 40 pots), and the 3 groups were given different water treatments, namely CK, T1 and T2. CK was normal irrigation in which the soil moisture level was maintained at 60% ~ 70%; T1 was moderate drought stress (soil moisture level 40% ~50%); T2 was super drought stress (soil moisture level 20% ~30%)[16]. The soil moisture level was controlled by weighing pots and adding water to bring it up to the desired level[17]. The sampling and measurement were conducted every 10 days, respectively on August 20, August 30, September 10 and September 20, four times in all. Each time we chose 5 plants from each treatment group to conduct the measurement of relevant indices.

1.2 Measurement

1.2.1 *Heights and base diameters* Plant heights were measured to the nearest 1 cm using a tape measure, and the base diameters were measured to the nearest 0.1mm using a vernier caliper.

1.2.2 *Root/shoot ratio* Based on drying method, the above-ground part and the underground part of the seedlings were dried in the oven at 80 ℃ until constant weight was obtained[18], and then weighed with a 1/10 000 analytical balance. The root/shoot ratio was the ratio of underground to above-ground dry biomass.

1.2.3 *Root surface area and specific root area* The root surface area was measured with the Canada Win-RHIZO root analysis system. The specific root area ($cm^2 \cdot g^{-1}$) was the ratio of the root surface area to the dry weight of the root.

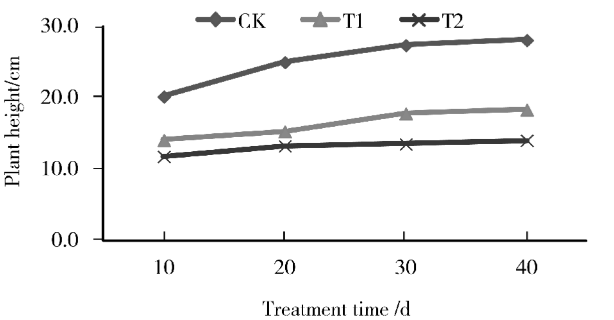
1.3 Data processing

The data were entered in Micro-Excel for collection of statistics and graphing, and analysis of variance was then conducted using SPSS software.

2 Results and Analysis

2.1 Growth in heights of mulberry trees at three levels of soil moisture

As Fig. 1 shows, during the same period, the average trees heights of T1 and T2 treatments were both lower than that of CK, and decreased significantly ($p < 0.05$) with increasing drought stress. As the treatment time proceeded, the more serious was the stress, and the more slowly the mulberry trees grew. The average rate of growth in height (cm/d) of CK in the three stages measured (10 ~ 20 d, 20 ~ 30 d, 30 ~ 40 d) were respectively 0.48, 0.23 and 0.08; for T1 these were respectively 0.12, 0.25, and 0.05; and for T2 were respectively 0.14, 0.03, and 0.05. It should be noted that the large incremental difference between T1/ T2 and CK developed during the first 10 days.

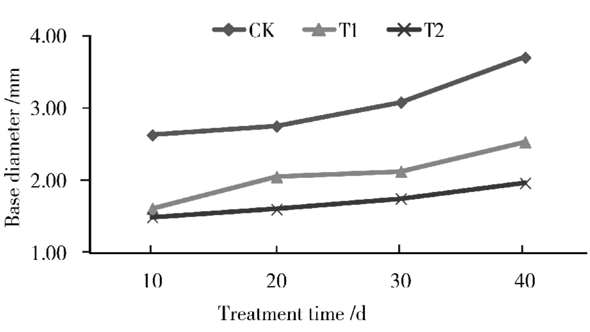


CK: normal irrigation; T1: moderate drought stress; T2: super drought stress

Fig. 1 Growth in height of mulberry trees over 40 days at three soil moisture levels

2.2 Base diameters of mulberry trees at three soil moisture levels

As Fig. 2 shows, the trees base diameters of T1 and T2 were both lower than that of CK during the same period, and decreased significantly ($p < 0.05$) with drought stress increasing. As the treatment time proceeded, the more serious was the stress and the more slowly the base diameters increased. The average base diameter growth rate ($mm \cdot d^{-1}$) of CK in the three stages that were determined (10 ~ 20 d, 20 ~ 30 d, 30 ~ 40 d) were respectively 0.012, 0.023 and 0.062; for T1 these were respectively 0.044, 0.007, and 0.041; and for T2 they were respectively 0.011, 0.015, and 0.021.

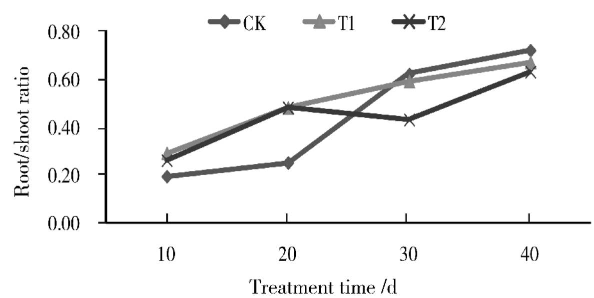


CK: normal irrigation; T1: moderate drought stress; T2: super drought stress

Fig. 2 Base diameters of mulberry trees during 40 days at three soil moisture levels.

2.3 Root/shoot ratio of mulberry trees at three soil moisture levels

As the treatment time proceeded, the overall root/shoot ratios of the mulberry trees of each treatment group increased (Fig. 3), but the root/shoot ratios of the three treatment groups showed differing trends. In the earlier stage (0 ~ 20 d), root/shoot ratios of T1 and T2 were both higher than that of CK. In the later stage (after 20 days), the root/shoot ratio of T2 decreased gradually, and then showed an increasing trend again. Statistical analysis showed that there were no significant differences between CK and T1, but the difference between CK and T2 in the later stage was significant.



CK: normal irrigation; T1: moderate drought stress; T2: super drought stress.

Fig. 3 Root/shoot ratio of mulberry trees at three soil moisture levels over 40 days

2.4 Root surface area and specific root area of mulberry trees at three soil moisture levels

As Fig. 4 shows, with drought stress increasing, the root surface area of mulbery trees in the various periods all decreased significantly. With prolongation of treatment time, the mulberry trees grew gradually, and the mulberry root surface areas in the various treatment groups all showed an increasing tendency (Fig. 4A). With drought stress increasing, the specific root area of mulberry trees in the various periods assessed showed a tendency to increase on the whole (Fig. 4B). The specific root areas in treatment T2 at the four measurement times (10th, 20th, 30th, 40th) were significantly increased 68%, 67%, 113% and 90% relative to those of CK during the same periods. With the elongation of treatment time, the specific root areas in the various treatment groups revealed decreasing tendencies overall. The differences were not statistically significant in the earlier stage, but there were significant differences in the later stage.

3 Discussion and Conclusion

Previous studies have shown that change in plant height and base diameter are two important plant morphological indices for measuring crop resistance to drought stress and that the growth of plants is inhibited under drought stress conditions. Furthermore, the more serious the stress, the more markedly the growth is inhibited[19-20]. In this study, there were significant effects of drought stress on the heights and base diameter of mulberry trees, and as drought stress increased the more obvious was the inhibition (Fig. 1 and Fig. 2). With elongation of treatment time, although the heights and base diameters in each treatment group still tended to increase, the growth rate decreased gradually.

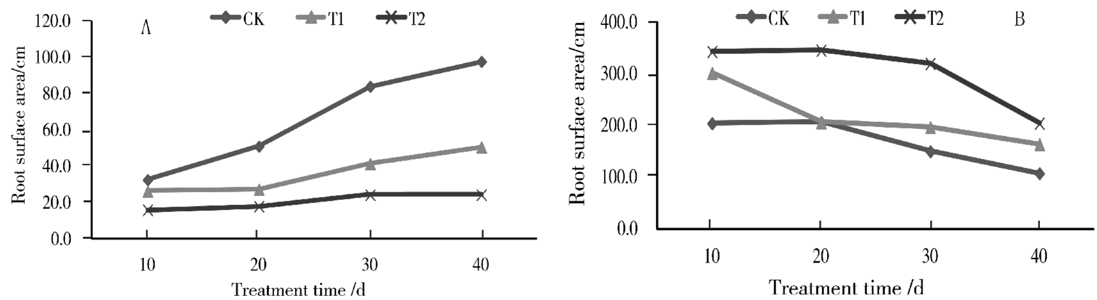


Fig. 4 Root surface area and specific root area of mulberry trees at three soil moisture levels over 40 days

The root system is the main organ of a crop that is responsible for absorbing water and mineral elements. Furthermore, the size, distribution and physiological activity of the roots largely determine the absorption of nutrients and water by the crop, thereby greatly influencing the growth, yield and quality of a crop. Many studies[20-27] have indicated that root morphological and physiological characteristics are closely related to plant drought resistance, and the root/shoot ratio, root surface area and specific root area are all important indices for measuring the development of seedling roots. These studies also show that trees with larger root/shoot ratio, root surface area and specific root area show stronger ability to absorb water and minerals. We concluded, with drought stress increasing and treatment time elongating, the root/shoot ratio and root surface area of mulberry trees both increased significantly, which corresponds with the conclusion of Xu et al[28]. Furthermore, with drought stress increasing, the specific root area also showed the tendency of significantly increasing. Accordingly, under the drought stress condition, plants can distribute a large proportion of limited photosynthetic products to the new root, in order to promote the root become longer and thinner, and increase the contact area with soil to expand the range of moisture absorption and strengthen the utilization of deep soil water[29-30]. In this way the plant reduces water consumption, avoids and endures water deficit, and so adapts to drought conditions[29-30].

The observations show pronounced effects of simulated drought stress, with the strongest effects developing within the first 10 days, and then increasing further subsequently. However, even in the drought stressed systems, growth variables increased with time, and no plants died, indicating adaptability to severe drought conditions. We therefore conclude that the mulberry plant has the ability to adapt to summer drought and soil drought in the hydro-fluctuation belt of the Three Gorges Reservoir Area, and is therefore a suitable tree species for revegetation experiments in the hydro-fluctuation belt.

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